



GUIDELINES FOR THE SUBMISSION OF SPECIMENS FOR RABIES TESTING

The Washington State Public Health Laboratory and King County Public Health Laboratory are the only laboratories in the state that perform rabies testing in animals. Animal bites to humans are required to be reported to the local health jurisdiction (to find a phone number for your local health jurisdiction, please visit: <http://www.doh.wa.gov/LHJMap/LHJMap.htm>). They will assess the situation and arrange for transport of the laboratory specimen when appropriate.

Laboratory testing

Rabies testing in animals is done using a direct fluorescent antibody (DFA) test; fresh brain tissue is required for this test. Three areas of the brain are tested (brainstem, cerebellum, and hippocampus). The head of the animal should be refrigerated and shipped as soon as possible after death. The bat or the head may be frozen as a last resort if shipment of the specimen must be delayed. Tissues should not be submitted in formalin.

Currently, there are no reliable, standardized ante-mortem (live animal) tests that can be used to confirm whether an animal is infected with rabies.

Prior to performing the rabies test, the microbiologist will determine the adequacy of the brain for testing. Conditions that are unacceptable for testing include: decomposition of the brain, crushing or gunshot into the brain, or unidentifiable or missing areas of the brain. Keeping the head cold helps slow deterioration of the brain. The amount of decomposition of a brain can be difficult to determine visually and is dependent on factors such as the ambient temperature and amount of time since death. Potentially decomposed or destroyed brains should be submitted to the laboratory for evaluation of whether they can be tested. Any animal that is excreting rabies virus in their saliva should have detectable virus in the brain by DFA examination.

Prior to shipping specimens, contact Communicable Disease Epidemiology at (206) 418-5500 or (877) 539-4344.

Test Results: Rabies laboratory results are usually available within one working day of receipt of the specimen.

Preparation of specimens for submission (animal heads and whole bats):

Bats should be submitted intact. Other animals should be decapitated and only the head submitted. Veterinarians can euthanize live bats. It is against the law to ship bats or any other animal alive.

Decapitation Supplies:

1. Sharp knife and sharpener (optional-sharp hacksaw, dehorner, shears, or brush cutters)
2. Protective clothing:
 - Waterproof gloves (disposable or disinfect after use)
 - Face mask
 - Safety glasses or goggles

- Optional coverall, waterproof apron (a large plastic garbage bag with holes for arms and head)
- 3. Cleaning supplies: Water, detergent, a disinfectant with virucidal activity (bleach), paper towels and plastic trash bags.

Decapitation Procedure:

- Only veterinarians, animal control officers, local health or wildlife officials, and others who have been appropriately trained and have rabies pre-exposure prophylaxis should remove animal heads. CAUTION: The brain, spinal cord, salivary glands, and saliva of the animal may contain rabies virus. If an exposure occurs during the process of removal of the head, it should be noted on the laboratory submission form for the animal.
- Do not use an axe or power saw because infected material may become aerosolized.
- Always wear protective gear (waterproof gloves, a facemask and eye protection) and exercise caution with the use of knives and other sharp objects during the procedure.
- Lay the animal on a surface and extend the neck. If you do it on a table you can hang the head over the edge with the neck exposed.
- Cut at a point several vertebrae below the head. This will ensure that brain stem is included in the specimen.
- Immediately package the head (see instructions below).

Clean up: Instruments and contaminated surfaces should be washed with detergent and water and then disinfected. Disinfect with a freshly prepared solution of bleach (1 pint per gallon of water), alcohol (40-70% ethanol), iodine (25 ppm) or quaternary ammonium (200 ppm) compounds. The disinfectant should remain in contact with the infected surfaces for a minimum of 10 minutes.

Packaging and Shipping instructions

The shipper is responsible for packaging specimens correctly and shipping them rapidly. Packages that are not correctly labeled may be returned to the sender by the shipping company. Shipping regulations changed on January 1, 2005; the packaging information below has been updated. People who package specimens for shipping are legally required to take a class in shipment of infectious substances; the class is available commercially or through Washington State Public Health Laboratories Training Program. For more information on the classes, please e-mail PHL@doh.wa.gov.

Shipping materials that conform to current regulations are available from local health jurisdictions.

Follow these directions to package a specimen for transport to the laboratory:

- a. The whole bat or head of the animal should be sprayed for fleas if needed and placed in a plastic bag. Seal the bag.
- b. Place the bag containing the bat or animal head inside a larger plastic bag. Seal this bag.
- c. The shipping containers obtained from your local health jurisdiction consist of a plastic screw-capped jar or metal can (secondary container), absorbent material for the secondary container, and an insulated shipping box.
 - i. Place bagged bats or small animal heads in the secondary container which then fits into the small box.

- ii. Larger animal heads require the white 10 liter metal drum that goes in the large beige with red and blue colored Air-Sea box.
- d. Place frozen ice packs between the secondary container and shipping box. Check ice packs for rips or tears which could leak gel material as it thaws. A leaking package may be rejected by a courier.
- e. Fill out the Rabies lab form (there should be a blank form inside the shipping box). Place the form in a ziplock bag and put the ziplock bag in the box but not in the secondary container with the specimen. Everything in the secondary container is considered as potentially infectious and it will be autoclaved after the lab testing is completed.

Information to accompany all specimens:

- Name, address, and phone number of the submitter
- Date of death and location where collected
- Species of animal
- Owner of animal - Name, address, and phone number
- Person(s) and/or animal(s) exposed; date of exposure
- i. The name of the person to contact with the results and a phone number where that person can be reached must be on this form. The contact person is usually a local health jurisdiction employee who will follow up with positive cases.
- f. The outside of the cardboard box should have a "Diagnostic Specimen" label next to a diamond shape with "UN3373" inside the diamond. Another label displays the names and addresses of the shipper and recipient.
- g. Specimens should be refrigerated, and shipped or delivered to the lab as quickly as possible (overnight FedEx or same-day bus service are commonly used transport methods). Always save the tracking number or bus bill so the package can be traced if it is lost.
- j. Horse or cow heads will not fit in the above packaging. When possible, remove the entire brain. A minimum of one-half of the cerebrum and full cross-sections of the cerebellum and the brain stem are needed. If it is not possible to remove the brain, wrap the entire head in at least three plastic bags, each one separately sealed. Several frozen ice packs can be placed inside the outer bag. Place the wrapped head in a cardboard box. Because this is not standard packaging, none of the shipping companies nor Greyhound bus can ship the package. It must be delivered to the laboratory by vehicle.

Routine submissions can be received at the laboratory between 8AM and 5PM Monday - Friday. After hours submissions can be arranged under certain circumstances (pending a decision about rabies PEP) through Communicable Disease Epidemiology (206) 418-5500 or (877) 539-4344.

Specimens should be delivered to:

Washington Public Health Laboratories
 Attention: Virology
 1610 NE 150th Street
 Shoreline, WA 98155

Rabies laboratory phone number: (206) 418-5458